

The Association between Ocular Problems and Serum Testosterone, Prolactin and Thyroglobulin Concentrations in Delayed Phase of Sulfur Mustard Exposure

Hassan Ghasemi^{1,2}; Nayere Askari³; Sakine Moaiedmohseni¹; Soghrat Faghihzadeh⁴; Susan Kabudianian Ardestani⁵; Elham Faghihzadeh¹; Tooba Ghazanfari^{1*}

1. Immunoregulation Research Center, Shahed University, Tehran, I.R. Iran

2. Dept. of Ophthalmology, Shahed University, Tehran, I.R. Iran

3. Dept. of biology, Faculty of Basic Sciences, Shahid Bahonar University of Kerman, Kerman, I.R. Iran.

4. Dept. of Biostatistics and Social Medicine, Zanzan University of Medical Sciences, Zanzan, I.R. Iran

5. Institute of Biochemistry and Biophysics, Tehran University, Tehran, I.R. Iran

KEYWORDS

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ABSTRACT

Background and objective: Aside from direct toxic effects, Sulfur Mustard (SM) induced serum hormone abnormalities may aggravate ocular complaints, including Ocular Surface Discomfort (OSD) (burning, itching, and redness), dry eye sensation, photophobia, blurred vision, foreign body sensation, and pain. The aim of the current study was to investigate the possible association of ocular complaints with serum hormone concentrations in chronic phase of Sulfur Mustard (SM) exposure.

Methods: As a part of Sardasht Iran Cohort Study (SICS), 372 SM-exposed patients and 128 non-exposed participants were enrolled. Ocular complaints and ocular surface biomicroscopic conditions and serum hormones were compared.

Results: The exposed with tearing group had significantly higher mean serum levels of testosterone and prolactin (ng/mL) than controls (5.75 vs. 4.75, $P=0.031$; 11.71 vs. 8.42, $P=0.009$). The exposed with OSD group had significantly higher mean serum levels of prolactin than controls (12.48 vs. 6.90, $P=0.002$). The exposed with photophobia group had significantly higher mean serum levels of testosterone than the matched exposed (6.25 vs. 5.65, $P=0.013$). The exposed with blurred vision group had significantly higher mean serum levels of Thyroglobulin (Tg) (ng/mL) than the matched exposed (65.73 vs. 32.6, $P=0.003$).

Conclusion: Higher mean serum levels of testosterone (in exposed with tearing and photophobia) and prolactin (in exposed with tearing and OSD) may play protective roles against SM effects. Higher mean serum levels of Tg may deteriorate the tear film integrity and optical surface, which causes blurred vision. In the chronic phase of SM toxicity, some ocular surface problems are associated with alterations in the serum concentrations of testosterone, prolactin, and Tg.

Corresponding information: Tooba Ghazanfari, Immunoregulation Research Center, Shahed University, Tehran, I.R. Iran. Tel: +98-2188964792, Fax: +98-2188966310, E-mail: tghazanfari@yahoo.com; ghazanfari@shahed.ac.ir

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Introduction

Mustards in the forms of liquid or vapor are very persistent agents and in acute phase of exposure cause ocular problems, such as irritation, photophobia and decreased visual acuity, pulmonary edema and respiratory distress, and skin

lesions similar to first- or second-degree burns. Some of the clinical problems may continue for many years and even later and may lead to cancer (1). Local or systemic autoimmune responses may, however, be involved in the pathogenesis of acute or late human corneal involvement or animal

models of mustard gas keratitis (2). Except some palliative interventions, there are no effective and appropriate therapies against devastating ocular injuries induced by vesicant chemical agents (3). Limited effective medications have enforced researchers to test a variety of different treatments, such as immunological, chemical and/or herbal interventions (4-7). Aside from these serious side-effects, Sulfur Mustard (SM) causes many alterations in serum hormone concentrations during different phases of exposure (8). Under normal conditions, serum hormones influence the integrity of ocular surface and their abnormalities may cause ocular complaints, such as tearing, OSD) (burning, itching, and redness), dry eye sensation, photophobia, blurring of vision, foreign body sensation, and pain.

Sex hormones and the endocrine system hormones, including androgens, estrogens, prolactin, luteinizing hormone, and follicle-stimulating hormones play significant roles in the physiology and pathophysiology of the lacrimal glands in both genders of humans (9). Subjective symptoms related to tear production and stability in OSD, such as dry eye sensation, redness, and inflammation, are significantly related to periodic serum hormone fluctuations during the menstrual cycle. This is especially related to follicular phase and estrogen peak (10). Dry eye condition is particularly associated with postmenopausal ages in females. Decrease in serum and local levels of sex hormones are the basic pathogenic features of tear film hyperosmolarity, ocular surface and lacrimal gland inflammation, and dry eye disease, especially in females (11). Therefore, aside from traditional ways of replacement of artificial tears, sodium hyaluronate drops, topical cyclosporine A, autologous serum, and hormonal therapy might play an important role in the treatment of dry eyes in such patients (12). Phytoestrogen supplementation significantly decreased tear osmolarity, increased tear production, and improved problems induced by dry eye syndrome in postmenopausal females (13). Treatment with combined esterified estrogen and methyltestosterone may be effective for various types of dry eye syndromes (14).

Pituitary prolactin and prolactin-like hormones secreted by human lymphoid tissue and peripheral

mononuclear cells have been known as an immunomodulator in the recent years (15). Tear protein patterns are expressed under control of intraocular pressure that by themselves are under control of tear proteins, including prolactin-inducible protein (16). Inducing a Th2 immune response using cDNA encoding the human thyrotropin receptor in mice, caused an increase in total thyroxine (T4) and suppression of *Thyroid Stimulating Hormone* (TSH) levels, and finally led to the appearance of ocular surface disorders and orbital manifestations of Graves' ophthalmopathy (17).

Direct toxic effects of SM on ocular surface have been known for many years. As mentioned, various serum hormones including testosterone, prolactin, and thyroglobulin play constructive roles on ocular surface integrity. The aim of this study was to evaluate the possible association or enhancement of ocular problems induced by SM with alterations in serum hormone concentrations during the chronic phase of SM toxicity.

Materials and Methods

Study Design and Participants

This study was part of the Sardasht Iran Cohort Study (SICS). In this study, 372 documented SM-exposed male patients as cases and 128 non-exposed male participants as controls were enrolled and assessed by an ophthalmologist. All participants gave informed consent before entering the study. Both groups were matched for confounding variables. Demographical variables, such as height, weight, Body Mass Index (BMI), systolic and diastolic pressures, history of serious diseases and their durations, population size, weather, ethnicity, economic, distribution of gender and age, clinical variables, ethical code and approval date, equipment calibration, applied apparatuses, chemical kits, solutions and companies properties, criteria used for measuring variables, inclusion and exclusion criteria, sample size, expected power, sampling strategy, software and method of statistical analysis, have previously been explained (18, 19).

Ethical Considerations

The study was approved by the Ethical Committee of the Board of Research Ethics of Janbazan Medical and Engineering Research Center

(JMERC), the Board of Research of the Ministry of Health and Medical Education and Shahed University. Volunteers, who signed an informed consent, were recruited.

Ophthalmic examination

A thorough ocular history was taken, an eye examination was performed, and serum hormonal parameters were evaluated in all participants. Any ocular complaints, such as tearing, Ocular Surface Discomfort (OSD) (burning, itching, and redness), dry eye sensation, photophobia, blurring of vision, foreign body sensation, and pain, were recorded. The bio-microscopic conditions of lids, tear meniscus layer, bulbar conjunctiva, limbal tissue, cornea, and anterior segment were examined by a slit lamp (Nidek model, Gamagori, Japan).

Serum sampling

Peripheral blood samples were taken in the morning at a fasting state using Vacutainer tubes (BD Biosciences). After clotting, the sera were separated by 20 minutes of centrifugation at 2000×g and 4°C, aliquoted and stored at -80°C until use.

Measurement of Thyroid Hormones

Serum hormonal parameters, including testosterone, Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), prolactin, T4, triiodothyronine (T3), TSH, Free Thyroxine Index (FTI), free T3 index, Thyroglobulin (Tg) and Thyroglobulin antibody (TgAb), and anti-Thyroid Peroxidase (anti-TPO) antibodies were measured in both groups. Furthermore, T3, T4, and TSH were measured by the Enzyme-Linked Immunosorbent Assay (ELISA) method (dbc, Canada). Intra- and inter-assay Coefficients of Variations (CV) were 3.3% and 6.2% for T4, 12.3% and 10.4% for T3, and

3.9% and 7.1% for TSH, respectively. According to the manufacturer's instructions, the normal range was 0.7 to 2.1 ng/mL for T3, 4 to 12 µg/dL for T4, and 0.3 to 5 µIU/mL for TSH. Anti-thyroglobulin (anti-Tg) and anti-thyroid Peroxidase (anti-TPO) concentrations were measured by ELISA (Radim, Pomezia, Italy). Values of anti-TPO, up to 100 IU/mL and anti-Tg, up to 150 IU/mL were considered normal. The inter- and intra-assay CVs were 7.2% and 6.5%, respectively.

Statistical Analysis

Statistical analysis was performed using SPSS-PC (SSPS for Windows, version 16.0, Chicago) package program. Values are given as means (±SD). Between-group comparisons were performed by the Mann-Whitney U-test and t test, and within-group comparisons were performed by Wilcoxon signed-rank test and paired t test. Pearson's correlation coefficient was used to detect linear correlation in the total group. P values of less than 0.05 were considered statistically significant.

Results

Exposed subjects with tearing had significantly higher mean serum levels of testosterone and prolactin compared to matched controls (P=0.031 and P=0.009, respectively) (Tables 1 and 2).

Exposed participants with OSD had significantly higher mean serum levels of prolactin than matched controls (P=0.002) (Table 3).

The exposed subjects with photophobia had significantly higher mean serum levels of testosterone than their matched exposed group without this problem (P=0.013) (Table 4).

Table 1. Mean Serum Concentration of Testosterone (ng/mL) in Sulfur Mustard Exposed Patients with Tearing Compared With Matched Controls

Study Group	Tearing	Testosterone							
		N	Mean	SD	Median	Q1	Q3	P-value ^a	P-value ^b
Control	No	92	5.91	3.04	5.85	3.70	7.60	0.052	0.946
	Yes	32	4.75	2.35	4.85	2.50	6.45		0.031
Exposed	No	235	5.93	2.12	6.20	4.70	7.50	0.466	
	Yes	116	5.75	2.28	6.30	4.05	7.50		

Exposed subjects with tearing had significantly higher mean serum levels of testosterone than matched controls (P=0.031).

P-value^a: Comparison of participants within each group (t-test)

P-value^b: Comparison of participants between groups (t-test)

Table 2. Mean Serum Concentration of Prolactin (ng/mL) in Sulfur Mustard Exposed Patients with tearing compared with Matched controls.

Study Group	Tearing	Prolactin ng/ml							
		N	Mean	SD	Median	Q1	Q3	P-value ^a	P-value ^b
Control	No	95	10.93	8.82	8.50	6.20	11.95	0.116	0.057
	Yes	33	8.42	3.78	7.70	5.60	9.90		0.009
Exposed	No	246	12.97	8.88	10.50	7.30	15.70	0.164	
	Yes	127	11.71	6.91	10.10	7.10	14.40		

Exposed subjects with tearing had significantly higher mean serum levels of prolactin than matched controls (P=0.009).

P-value^a: Comparison of participants within each group (t-test)

P-value^b: Comparison of participants between groups (t-test)

Table 3. Mean Serum Concentration of Prolactin (ng/mL) in Sulfur Mustard Exposed Patients With Ocular Surface Discomfort Compared With Matched Controls.

Study Group	OSD	Prolactin ng/ml							
		N	Mean	SD	Median	Q1	Q3	P-value ^a	P-value ^b
Control	No	101	11.14	8.59	8.60	6.60	12.30	0.016	0.137
	Yes	25	6.90	2.38	6.30	5.45	7.85		0.002
Exposed	No	261	12.59	8.29	10.50	7.00	15.65	0.907	
	Yes	101	12.48	8.34	9.90	7.30	13.70		

Exposed subjects with OSD had significantly higher mean serum levels of prolactin than matched controls (P=0.002).

OSD=Ocular surface discomfort (burning, itching and redness).

P-value^a: Comparison of participants within each group (t-test)

P-value^b: Comparison of participants between groups (t-test)

Table 4. Mean Serum Concentration of Testosterone (ng/mL) in Sulfur Mustard Exposed Patients with Photophobia Compared With the Exposed Group without Photophobia

Study Group	Photophobia	Testosterone							
		N	Mean	SD	Median	Q1	Q3	P-value ^a	P-value ^b
Control	No	98	5.41	3.05	5.15	2.90	7.30	0.139	0.422
	Yes	26	6.36	2.20	6.90	4.50	8.20		0.813
Exposed	No	223	5.65	2.16	5.90	4.10	7.40	0.013	
	Yes	128	6.25	2.15	6.50	5.05	7.60		

Exposed subjects with photophobia had significantly higher mean serum levels of testosterone than exposed without photophobia (P=0.013).

P-value^a: Comparison of participants within each group (t-test)

P-value^b: Comparison of participants between groups (t-test)

Exposed participants with blurring of vision had significantly higher mean serum levels of Tg than

matched exposed without this problem (P=0.003) (Table 5).

There were no important levels of missing data to be effective on final analysis and conclusion.

Table 5. Mean Serum Concentration of Thyroglobulin (Tg) (ng/ml) in Sulfur Mustard exposed patients with Blurring of Vision Compared With Exposed Without Blurring of Vision

Study Group	Blurring of Vision	Tg							
		N	Mean	SD	Median	Q1	Q3	P-value ^a	P-value ^b
Control	No	80	14.60	16.62	9.20	4.90	18.00	0.232	0.054
	Yes	48	23.90	66.05	10.00	6.70	19.00		0.067
Exposed	No	214	32.60	86.03	10.80	5.60	19.00	0.003	
	Yes	159	65.73	153.40	11.80	6.70	31.00		

Exposed subjects with blurring of vision had significantly higher mean serum levels of Tg than matched exposed without this problem (P=0.003). The Tg level in the exposed group with blurring of vision was higher than the upper limit of normal value.

Tg= Thyroglobulin

P-value^a: Comparison of participants within each group (t test)

P-value^b: Comparison of participants between groups (t test)

Discussion

The findings of this study showed that there are associations between tearing and serum concentration of testosterone and prolactin and between OSD and serum concentration of prolactin in SM-exposed patients compared with matched controls. On the other hand, there were associations between exposed subjects with photophobia and serum concentrations of testosterone and exposed cases with blurring of vision and serum concentration of Tg compared with matched exposed.

Under normal conditions, testosterone down-regulates the genes that induce meibomian gland keratinization and thus prevents from meibomian gland dysfunction, lid margin abnormalities, and OSDs (20). Androgens, including testosterone, dihydrotestosterone, dehydroepiandrosterone, dehydroepiandrosterone-sulfate, and 17 beta-estradiol (but not progesterone) inhibit interleukin-1 beta-induced nitric oxide (NO) production, which is part of the ocular surface protection system against dry eye syndrome (21). In an animal study, Luo et al. showed that decreased serum androgens, especially testosterone in castrated male rabbits

caused lacrimal gland epithelial atrophy, mucus and acinar cells loss, significant decrease in the number of conjunctival goblet cells, and eventually a decrease in quantity and quality of tear secretion, and finally tear film instability (22). In one study on SM-exposed patients, plasma levels of Follicle-Stimulating Hormone (FSH) increased (23, 24), yet plasma levels of Luteinizing Hormone (LH) and testosterone remained unchanged (24). In one study conducted during the acute phase of exposure by Azizi et al., SM exposed casualties had significantly lower serum androgen levels accompanied with decreased responsiveness to gonadotropin-releasing hormone (GnRH) during the first 5 weeks, yet these values returned to normal 3 months later (25). Also, another study showed that during the first 5 weeks after SM exposure, the total and free serum testosterone levels decreased significantly, yet returned to normal, 3 months later. Sperm count significantly decreased within 3 to 9 years after exposure (26). In another study, most exposed patients had lower sperm counts and higher FSH levels than normal controls, 1 to 3 years after SM exposure (27).

In the present study, the researchers encountered higher mean serum levels of testosterone in patients with tearing compared with matched controls and in exposed subjects with photophobia compared with the exposed without photophobia. This elevation in serum concentration of testosterone may act as a reactive and perhaps protective mechanism against OSD and photophobia induced by SM in the chronic phase. In a rabbit model of Sjogren's syndrome-associated dry eye, analysis of tears collected from the ocular surface showed down regulation of prolactin-inducible protein (28). Lactoferrin and lacritin genes are directly related to lacrimal function, and other than cholinergic, adrenergic, vasoactive intestinal polypeptide, and purinergic genes for control of lacrimal function, androgen and prolactin receptors are also expressed in accessory lacrimal glands of Wolfring (29). Considering the protective roles of prolactin in OSD, especially in tear production, higher mean serum levels of prolactin in patients with tearing and OSD may be a protective mechanism against OSD induced by SM in the chronic phase. Based on the protective roles of hormones, investigators proposed hormonal therapy for reducing SM toxicity (30). On the other hand, high mean serum levels of prolactin in SM-exposed patients with skin cherry angioma reflect angiogenic activity of this hormone, which may also play an active role in late corneal angiogenesis in severely SM-exposed patients (31).

Nearly in all autoimmune disorders, such as primary Sjogren's syndrome, thyroid-related antibodies are significantly increased, suggesting a close relationship between immune-mediated disorders and autoimmune thyroid diseases (32). Furthermore, primary Sjogren's syndrome has been suggested as a predisposing factor for the development of autoimmune thyroiditis (33). Alteration in serum levels of thyroid hormones in SM-exposed patients is similar to changes seen in burn trauma. In the first week, serum levels of free T4 and T3 decreased, while reverse T3 (rT3) increased. Serum hormones concentrations were unchanged until the fifth week after injury, except for an increase in Free plasma Thyroxine Index (FT4I) and a decrease in serum TSH (34). On the other hand, higher mean serum levels of Tg in exposed subjects with blurring of vision compared

to those exposed without blurring of vision may be an accelerating factor for deterioration of tear film integrity and optical surfaces of the eyes and may cause blurring of vision in the chronic phase of SM exposure. There are some studies reporting the existence of Tg in orbital tissues of patients with thyroid-related eye disorders, which is transmitted through the lymph to the orbital tissues (35). Also, it has been shown that this Tg has a thyroid origin and a positive slope exists between serum Tg and the orbital tissues (35). This finding may suggest that an increase of Tg in orbital tissues may be a reflection of its systemic increase.

This is the first study evaluating the effects of serum testosterone, prolactin, and thyroglobulin disturbances induced by SM on ocular surface via tear section. Difficult sampling and low tear secretions in this group of patients preclude greater numbers of tear hormones assessments.

In conclusion, some of the ocular problems, such as tearing, photophobia and blurring of vision are associated with alteration in serum concentrations of some serum hormones and biochemicals, especially testosterone, prolactin, and Tg in patients with chronic phase of SM toxicity. Although these associations may be accidental, yet further investigations may prove their clinical importance in the future and add further information to the medical literature.

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Conflict of interest

The authors report no conflict of interest in this study.

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